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INTRODUCTION

One of the last obstacles preventing the establishment of shrimp aquaculture as an industry is the closing of the life cycle of penaeid shrimp, especially the white shrimp, in the laboratory under controlled conditions. Another goal that must be achieved is the refinement of such techniques so that maturation technology can be transferred successfully. Once techniques have been refined, the commercial qualities of penaeid shrimp can be improved through genetics.

There have been a number of experiments designed to close the life cycle of penaeid shrimp in the laboratory. Johnson and Fielding (1956) reported the first successful maturation and spawning (with fertilized eggs) of the white shrimp *Penaeus setiferus* in ponds. Conte et al. (1977) matured *P. setiferus* and *P. stylirostris* in ponds. However, there was no hatching of the eggs, and there was no spawning of *P. setiferus*. Brown et al. (1979) successfully matured and spawned *P. setiferus* continuously over a three-month period producing 4,334,000 unfertilized eggs by controlling photoperiod, temperature and diet. In addition, Aquacop (1975, 1979) has successfully matured and spawned *P. stylirostris* and *P. vannamei*, producing variable eggs in a tropical environment. There are also two companies presently maturing and spawning *P. stylirostris* in captivity, i.e., Ralston Purina and Marine Culture Enterprises. Ralston Purina has been producing since 1975 and is currently producing at a commercial level (Persyn, Ralston Purina Co., Crystal River, Fla.).

The initial successes in penaeid shrimp maturation have been experiments conducted on the grooved or brown shrimp with the exception of *P. setiferus* (Johnson and Fielding 1956). Hudinaga (1942) reported on the maturation and spawning of *P. japonicus*, but no further results on this species were published until recent years. Moore et al. (1974) reported on the maturation and spawning of *P. californiensis* in captivity but drew no conclusions from their studies; however, they noted decreased egg production of laboratory matured animals in comparison to mature females from the natural environment. Similar maturation studies were conducted by Aquacop (1975) in a tropical environment on *P. merguiensis* (3,000 eggs/spawn for small female and 20,000 eggs/spawn for a large female), *P. aztecus* (10-20,000 eggs/spawn), and *P. japonicus* (40,000 eggs/spawn). Laubier-Bonichon and Laubier (1976) and Laubier-Bonichon (1978) conducted further experimentation on *P. japonicus* in which the environmental parameters of photoperiod and temperature were rigorously controlled, resulting in the maturation of *P. japonicus* in three months, producing viable eggs. *P. monodon* has also been investigated for aquaculture by Aquacop (1976, 1977) and SEAFDEC (1976). Both groups have matured and spawned *P. monodon* successfully.

The majority of the successful experiments on the maturation and spawning of white shrimp *P. stylirostris* and *P. vannamei* have been conducted in a tropical environment (Aquacop 1975, 1979; Persyn, personal communication; Moore, personal communication). Very few studies of white shrimp maturation and spawning have been conducted in a temperate environment with the exception of Brown et al. (1979).

The objective of cooperative experiments conducted by the National Marine Fisheries Service, the University of Houston, and Texas A&M University was to mature and spawn *P. stylirostris* in a temperate climate by controlling temperature, photoperiod, and diet. *P. stylirostris* was selected because of the success other institutions have had in maturing

and spawning this species and its presumed fast growth rate in ponds. Additionally, these studies were undertaken to determine whether a production unit in a temperate climate can produce enough eggs and larvae to support a commercial aquaculture operation.

MATERIALS AND METHODS

Adult *P. stylirostris* were supplied by Maricultura, S.A., of Costa Rica and Marine Culture Enterprises, Puerto Peñasco, Mexico. *P. stylirostris* from Costa Rica were received on May 9, 1979, and from Mexico on June 15, 1979. The mean sizes were 51 g for males and 64 g for females. The animals were held in four 3.0 m diameter fiberglass tanks equipped with egg collectors as previously described (Brown et al., 1979) (Table 1). All maturation tanks were without substrate. (See schematic diagram of tanks and egg collectors in Fig. 1.)

Table 1. Number of Animals per Maturation Tank (MT) Feeding[a] and Ablation Schedule

MT #	Number of females	Number of males	Unilateral eyestalk ablation	Diet	
				Type	Time
1	11	15	-	Worms (dried food)	0800
2	16	15	+	Squid	1100
3	26	25	+	Worms	1400
4	24	24	+	Squid	1700[b]

[a]The animals in each maturation tank were fed at 2.5 to 3.0% body weight per day.

[b]A later feeding is made when possible.

Two of these tanks, maturation tank #1 (MT-1) and maturation tank #2 (MT-2) were stocked with the Mexican strain of *P. stylirostris* at 3.6/m^2 and 4.3/m^2, respectively. Maturation tank #3 (MT-3) and maturation tank #4 (MT-4) were stocked with Costa Rican *P. stylirostris* at 7/m^2. The lower stocking density of MT-1 and MT-2 occurred because of the high mortality of the Mexican strain of *P. stylirostris* in the laboratory. All tanks were supplied with a continuous flow of water ranging from 1.8 to 3 turnovers a day. The photoperiod was initially held at 12 h light and gradually increased to 14 h. The temperature of the inlet water was maintained at 29°C-30°C by Neslab[4] heat exchangers, and the salinity varied from 20 to 30 ppt. The diet and feeding regime remained essentially the same as that reported previously (Brown et al. 1979), with one exception, a compounded food was initially used but discontinued because of under-utilization by the shrimp and eventual fouling of the tanks.

[4]Trade names referred to in this publication do not imply endorsement of commercial products by the National Marine Fisheries Service, NOAA.

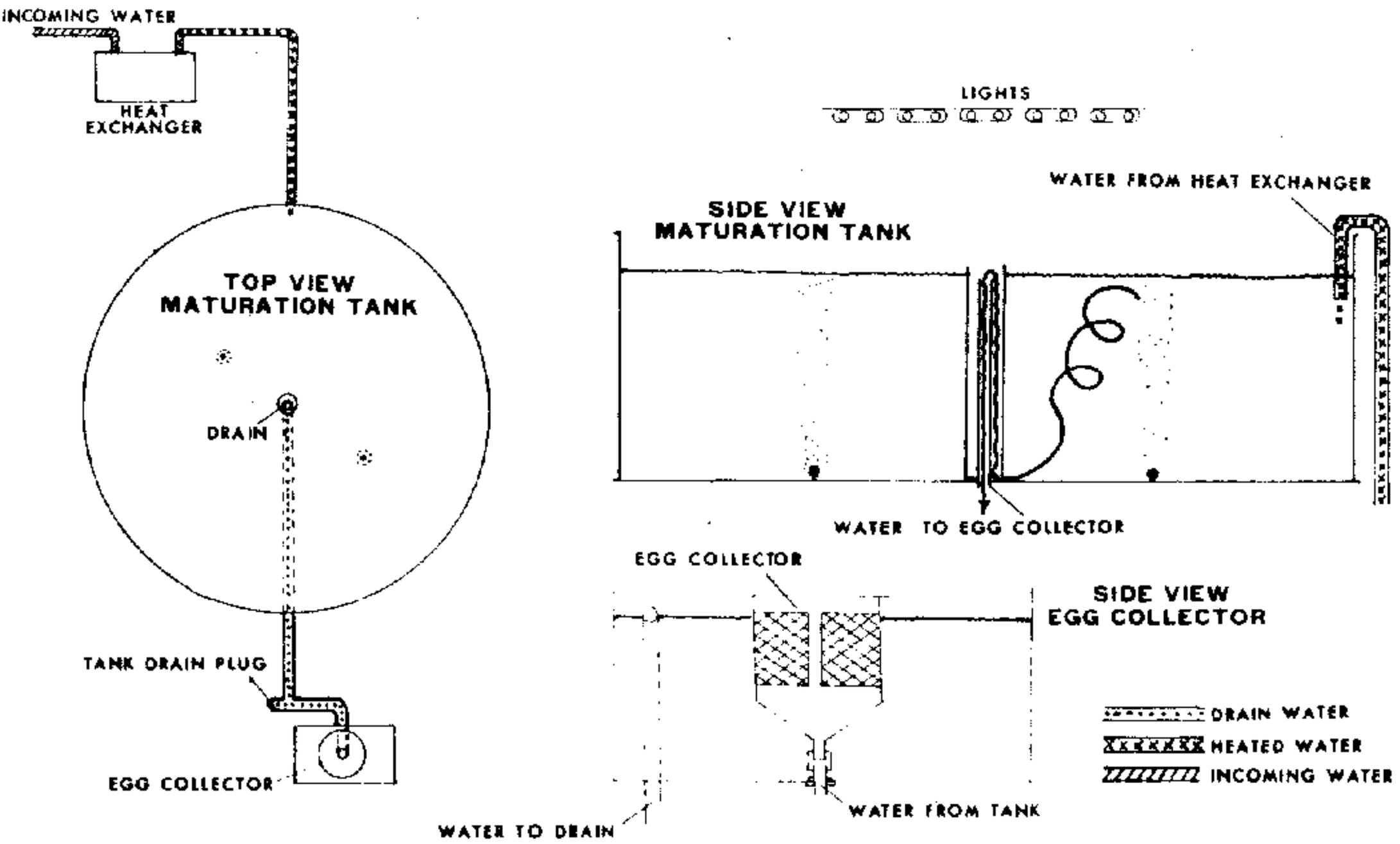

Figure 1. Schematic diagram of maturation system (National Marine Fisheries Service).

All females in MT-2, MT-3 and MT-4 were unilaterally ablated as previously reported (Brown et al. 1979) on June 20, 1979. The females of MT-1 were not ablated to determine whether *P. stylirostris* from Mexico would mature and spawn in our system without unilateral ablation, and if so, how egg production would compare with Mexican *P. stylirostris* that have been unilaterally ablated.

All females were checked daily by observing the shrimp in the tanks only. Female shrimp were handled only in transferring them from the maturation tanks to the spawning tanks. Mating activity (chasing) was observed to start around 1400-1500 h and continue until 2200-2230 h and later. Females with attached spermatophores (spawners were collected between 2100-2200 h and placed in spawning containers (400 and 500 liters) with airlift pumps (Mock, personal communication). Water temperature in the spawning tanks was maintained at 29-30°C with two aquarium heaters. The water of the spawning tanks was pretreated with EDTA[4] and the antibiotics Maracyn I[4] and Maracyn II[4]. Both antibiotics were used at a rate of one tablet per 10 gal of water before the addition of shrimp for spawning.

The eggs were allowed to hatch in the maturation laboratory and the nauplii were transferred to the hatchery for grow-out to the first stage postlarvae as a test of their viability. Counts of eggs, nauplii and protozoea were made by taking three to five 250 ml samples from spawning tanks after thorough mixing and extrapolating the average count per 250 ml to the volume of the spawning tanks. Since the hatchery could not handle all of the larvae produced, selected spawns were held in the spawning tanks until first stage protozoea as a check to insure that the larvae were capable of metamorphosis to protozoea. All of the larvae from spawns not taken to the hatchery and those held until first stage

protozoea were frozen for later biochemical analysis.

RESULTS

Ovarian development was observed approximately two weeks after unilateral ablation and the first eggs were collected July 28, 1979. There were a number of spawns observed in the egg collectors before the first large spawn on August 16, 1979. Some of these eggs were fertilized and some were not; however, animals with the spermatophore attached were selected for spawning after these initial observations (Table 2).

Table 2. Eggs Spawned in Maturation Tank and Collected in Egg Collectors

	Number of collections	Number of collections fertilized	Percent of collections fertilized	Total number of eggs
P. stylirostris (Mexico)	55	24	44	4×10^6
P. stylirostris (Costa Rica)	131	96	73	20×10^6

During the 190-day period, from August 16, 1979, to February 20, 1980, a total of 247 females (Costa Rican) with attached spermatophores were spawned producing 98 x 10^6 eggs, 55 x 10^6 nauplii with 56% hatching rate. The Mexican *P. stylirostris* produced a total of 7.4 x 10^6 eggs, 4.1 x 10^6 nauplii with a hatching rate of 55% from 19 females separated for spawning. There were approximately 9 to 10 spawns per female. This approximation is based upon 247 spawns and a total of 25 to 28 females in MT-3 and MT-4. Total production reached 105 x 10^6 eggs and 59 x 10^6 nauplii and a hatching rate of 56% (see Fig. 2-9) and from selected hatchery experiments, 746,000 postlarvae. Approximately 9-12% of the females were observed to have attached spermatophores (spawners) each night throughout the experiment.

The Costa Rican *P. stylirostris* produced approximately 397,000 eggs and 223,000 nauplii each spawn. The Mexican *P. stylirostris* produced 389,000 eggs and 216,000 nauplii each spawn. Four females with attached spermatophores were collected from the unablated Mexican *P. stylirostris* and 15 females were collected from ablated Mexican *P. stylirostris*.

Survival of the Mexican *P. stylirostris* was 18.5% for females and 63% for males in comparison to 48% female and 80% male survival for the Costa Rican *P. stylirostris*. Each animal molted approximately nine times during the 6-month 6-day period beginning August 16, 1979, to February 20, 1980.

Eighty-eight spawns were held in the maturation laboratory until the larvae reached first stage protozoea. Approximately 34 x 10^6 nauplii were held in this manner resulting in 25 x 10^6 protozoea with a survival rate of 74% (Table 3).

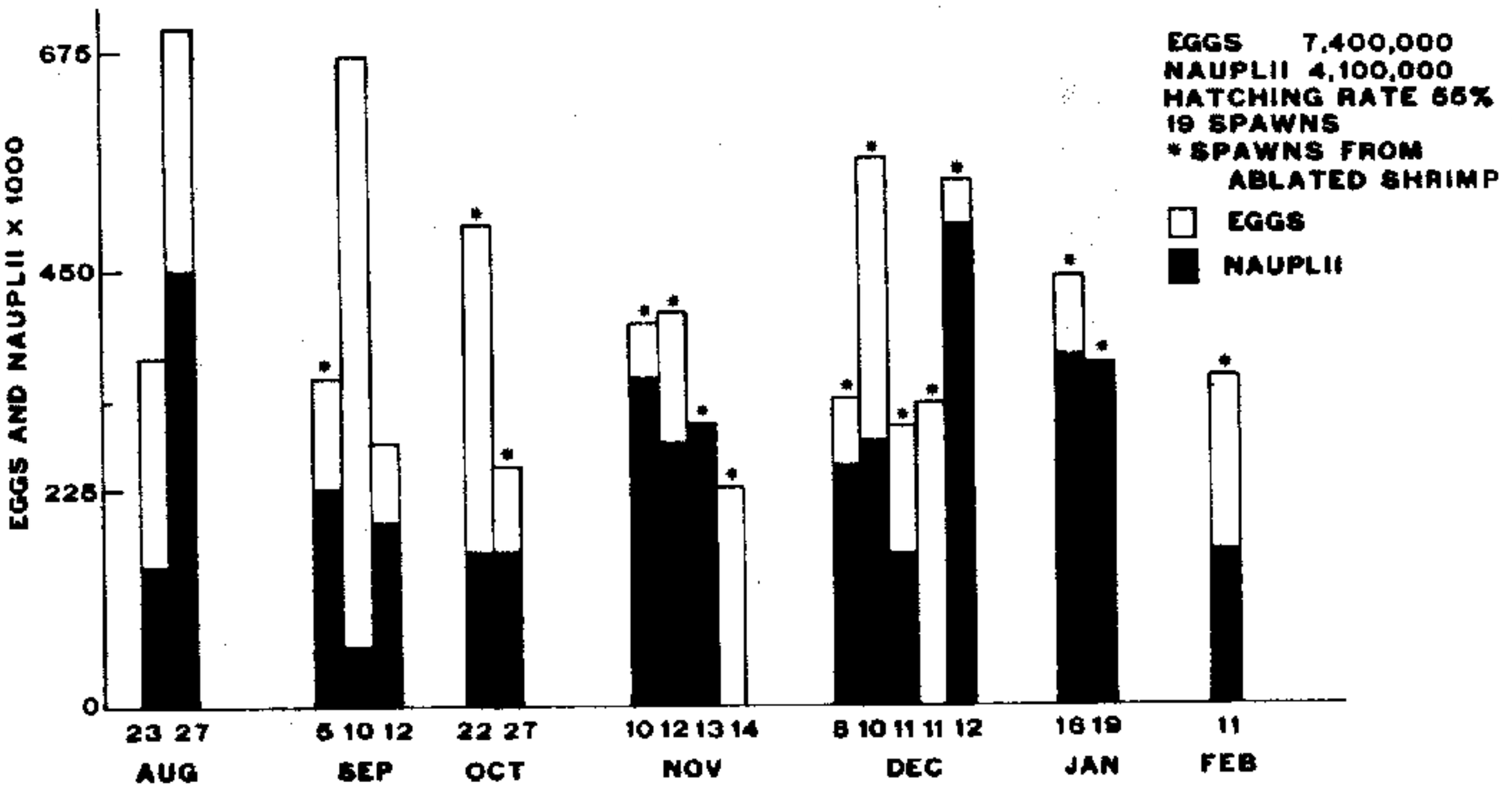

Figure 2. Maturation and spawning of *Penaeus stylirostris* (Mexico).

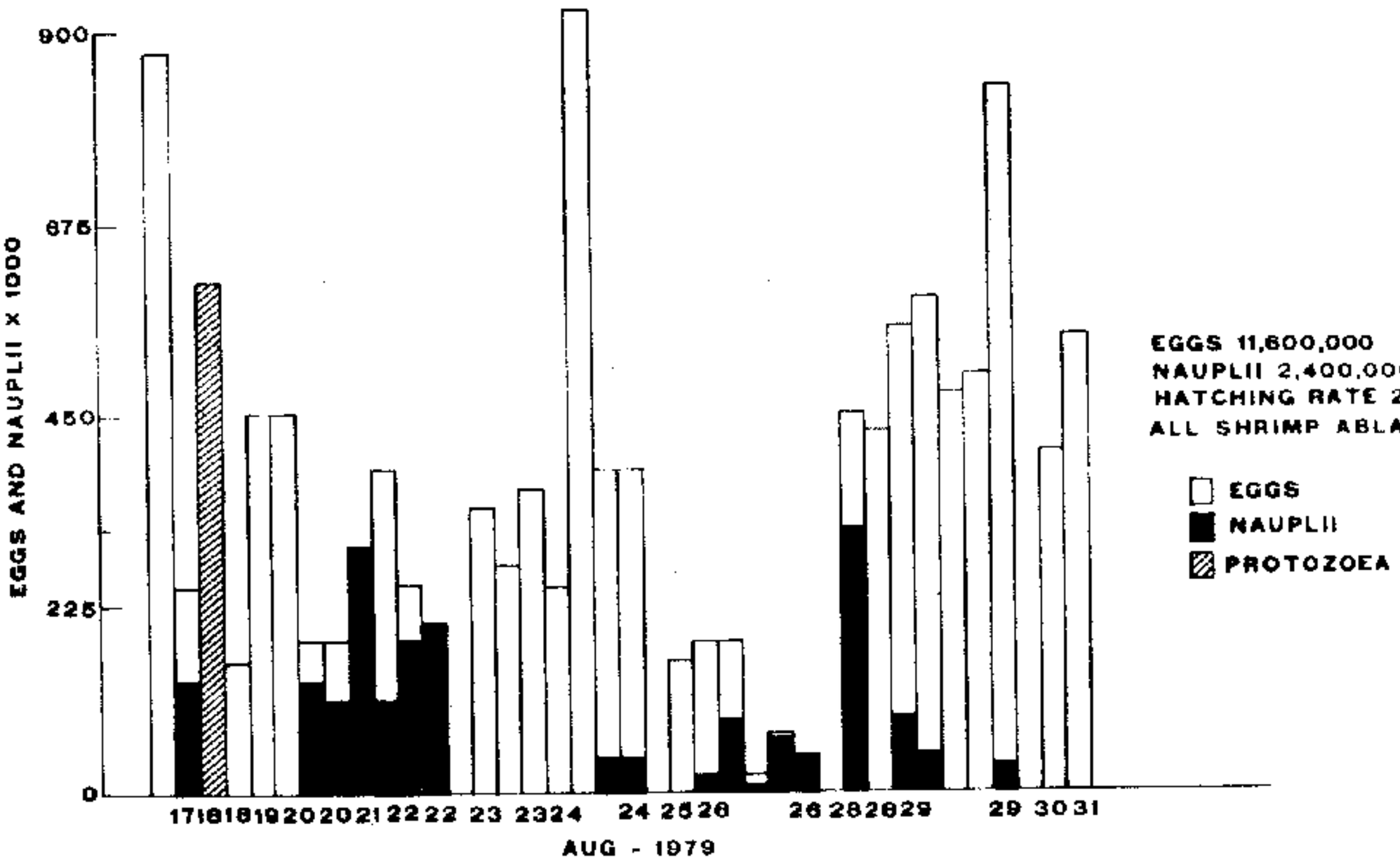

Figure 3. Maturation and spawning of *Penaeus stylirostris* (Costa Rica), August 1979.

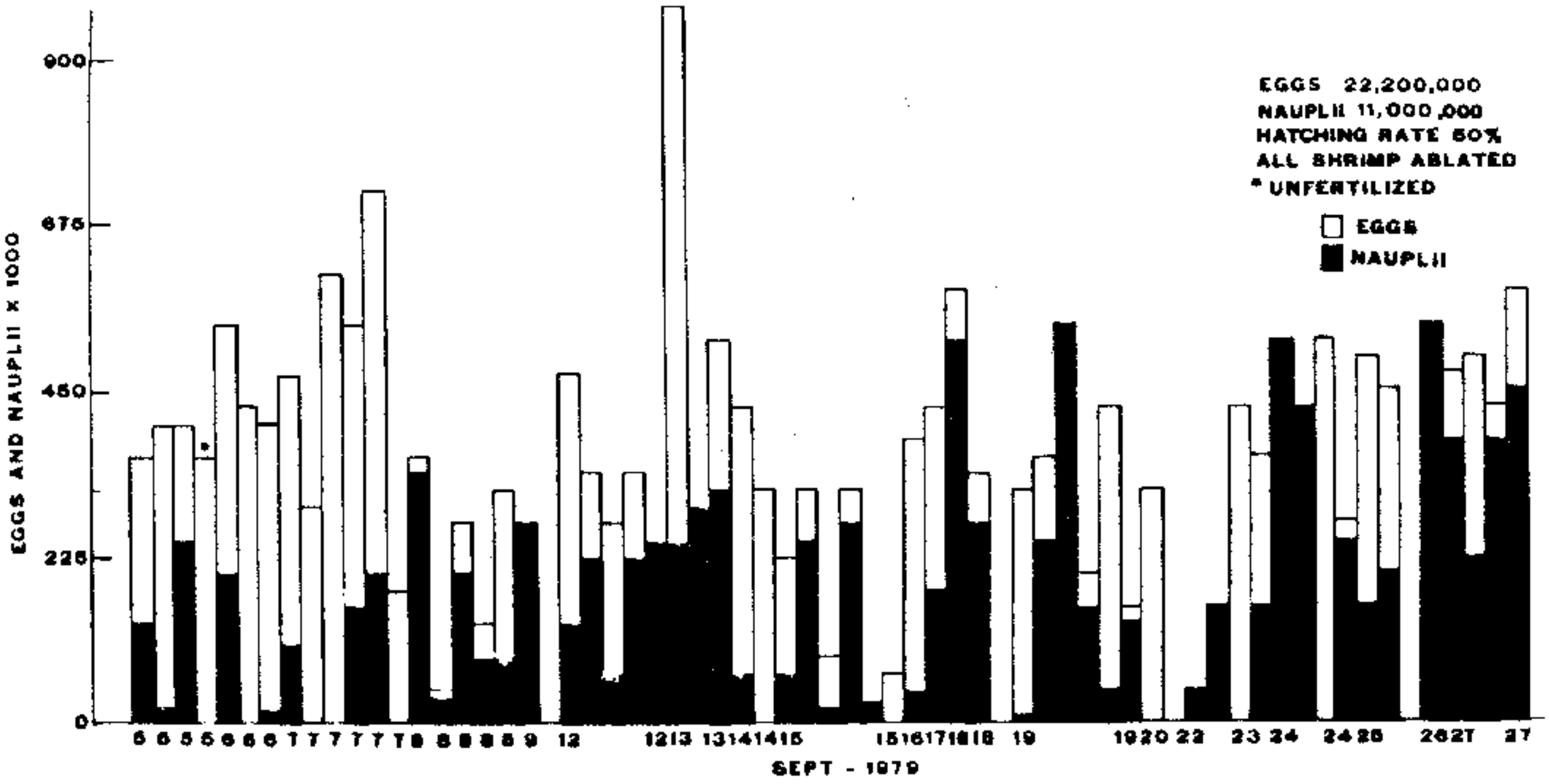

Figure 4. Maturation and spawning of *Penaeus stylirostris* (Costa Rica), September 1979.

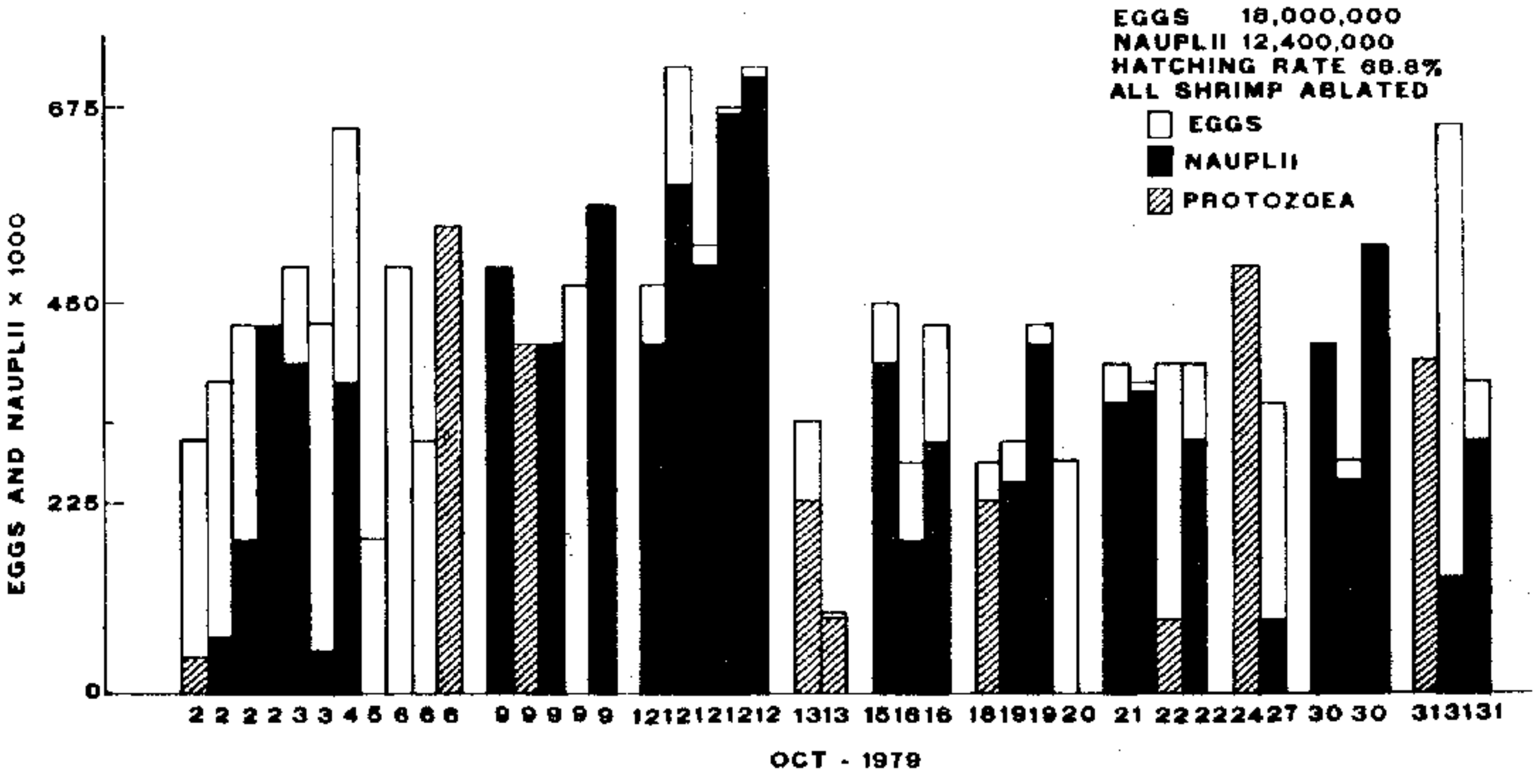

Figure 5. Maturation and spawning of *Penaeus stylirostris* (Costa Rica), October 1979.

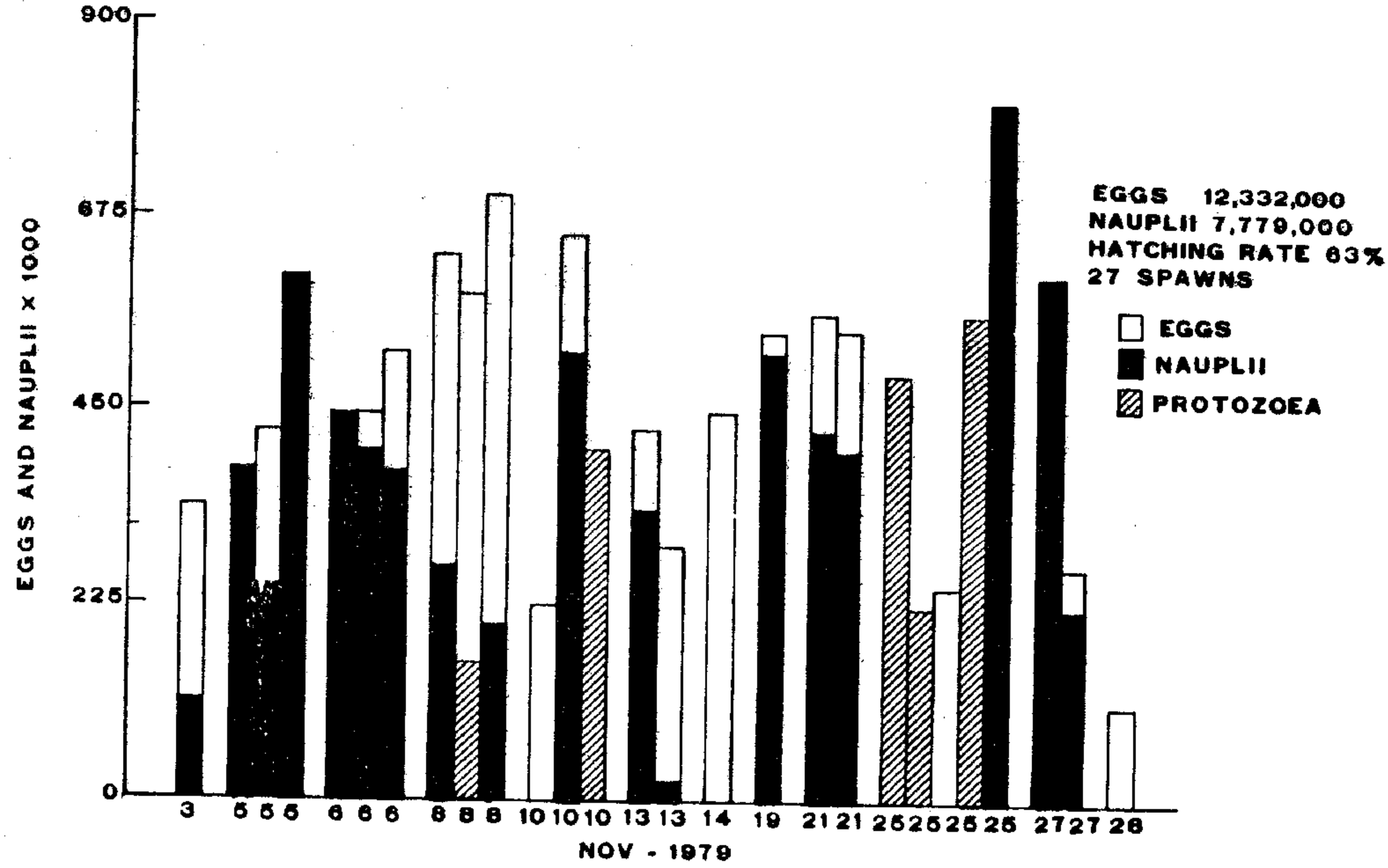

Figure 6. Maturation and spawning of *Penaeus stylirostris* (Costa Rica), November 1979.

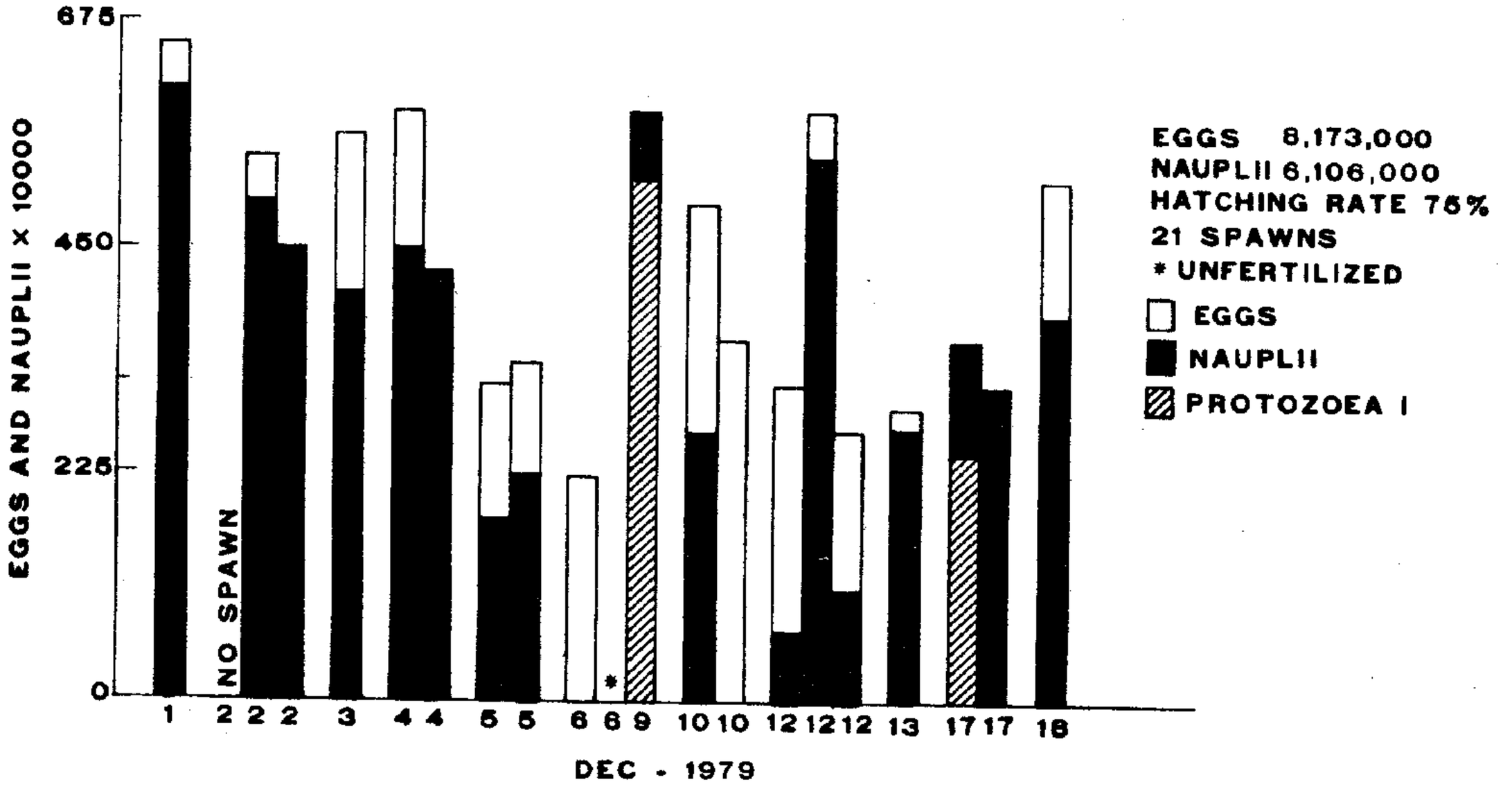

Figure 7. Maturation and spawning of *Penaeus stylirostris* (Costa Rica), December 1979.

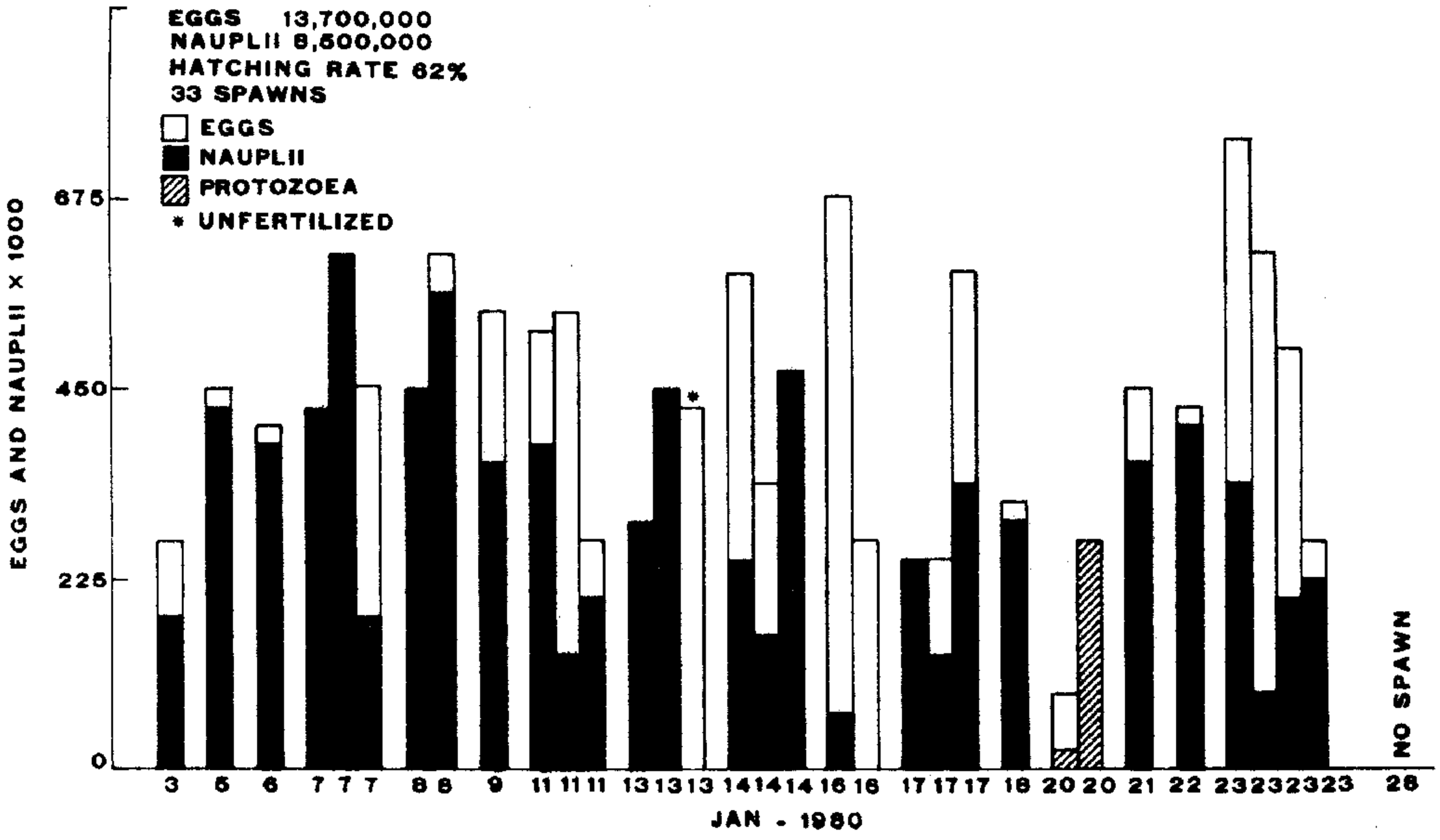

Figure 8. Maturation and spawning of *Penaeus stylirostris* (Costa Rica), January 1980.

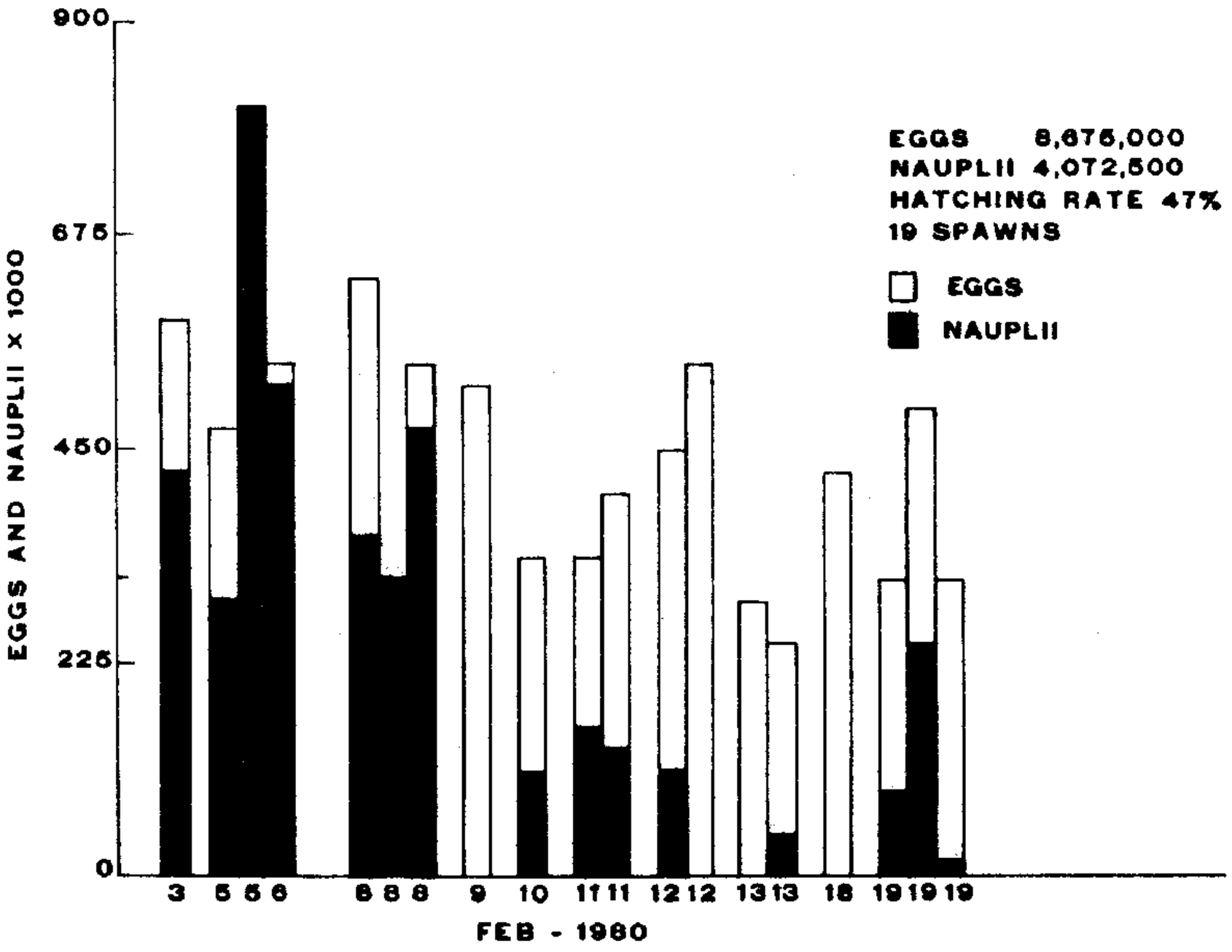

Figure 9. Maturation and spawning of *Penaeus stylirostris* (Costa Rica), February 1980.

Table 3. Survival of First Stage Nauplii to First Stage Protozoea

Month	Nauplii	Protozoea	Percent survival	No. of spawns
August				
September	4.8×10^6 [a]	3.9×10^6	81	18
October	7.5×10^6	6.2×10^6	83	23
November	12×10^6	7.3×10^6	61	22
December	5×10^6	4×10^6	80	11
January	4×10^6	3.3×10^6	72	14
Total	34×10^6	25×10^6		88

[a]Totals for August and September.

Throughout this study, eggs were observed in the egg collectors, indicating that all females with attached spermatophores were not taken out of the tanks on any given night. There were 186 spawns (or collections) from the maturation tanks, 65% of the spawns were fertilized (Table 2) resulting in 24×10^6 eggs.

DISCUSSION

Both strains of *P. stylirostris* adapted well to the tanks except for the high mortalities among the Mexican strain. Previous experiments with *P. setiferus* (Brown et al. 1979) indicated that a black color reduced the incidence of animals swimming into the sides of the tanks. The same tanks were used in the maturation of *P. stylirostris* with the same effect causing the animals to avoid colliding with the sides of the tanks.

Mating activity was noted in the maturation tanks before unilateral ablation. The mating characteristics of *P. stylirostris* from Costa Rica were very similar to those reported by Aquacop (1977) for *P. stylirostris* from Crystal River, Florida, and the Vera Cruz hatchery of Ralston Purina.

Interestingly, in the tropics, mating behavior occurs earlier on cloudy days and later on sunny days (Aquacop 1977, 1979). Costa Rican *P. stylirostris* started mating activity every day between 1400 and 1500 h regardless of external climatic conditions. The photoperiod was accidentally shifted as a result of a power failure and a concomitant shift in the initiation of mating activity resulted.

The hatching rate of eggs in the collectors was very poor. The reason for this resides in the fact that water from the maturation tanks exits through the bottom and with it debris, which attaches to eggs on the bottom of the collector (Fig. 1). Future plans include installing seawater outlets for the maturation tanks at the surface.

The hatching rate of eggs spawned in separate spawning tanks was appreciably better. The number of eggs and nauplii and the rate of hatching compare well with those reported by Aquacop (1979) of 100,000 to 250,000 eggs a spawn for *P. stylirostris* (Panama). In this study, *P. stylirostris* from Costa Rica averaged over 397,000 eggs a spawn and

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THE MATURATION AND SPAWNING OF *Penaeus stylirostris* UNDER CONTROLLED LABORATORY CONDITIONS

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ABSTRACT

Approximately 35 female *Penaeus stylirostris* from Costa Rica and Mexico held at the National Marine Fisheries Service, Galveston Laboratory, mated and spawned at least 247 times within a 190-day period producing over 98 x 10^6 eggs. Average fecundity was 397,000 eggs and each female spawned between 9 and 10 times. The hatching rate varied from 0-85% with an average around 50%.

Female shrimp were ablated and placed in 3 m diameter tanks at a sex ratio of 1:1. The salinity ranged from 20-30 ppt and water temperature was maintained at 29-30°C. The diet consisted of polychaete worms, squid and sometimes a pelleted food in the ratio of 1:2:1. The photoperiod was 14 h/day using standard fluorescent lighting. No natural sunlight was available to the shrimp.

Mating behavior was observed to start around 1400-1500 h and continued until 2200-2230 h and later. Female shrimp observed with a spermatophore were captured and placed in spawning tanks, and the water was treated with EDTA and Maracyn I and Maracyn II. Egg production and hatching rate were calculated for each spawning tank. Nauplii were transferred to the hatchery facility and viability determined for selected spawns. Survival to postlarvae varied considerably and no conclusions can be made at this time regarding the reasons for variability. Over 746,000 postlarvae were produced.

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those from Mexico produced over 389,000 eggs a spawn.

The controlled experimental conditions in this study supported the maturation and spawning of *P. stylirostris* producing viable eggs. It is significant that less than 30 females held in two 3.0 m diameter tanks produced 55 x 10^6 nauplii, with an average number of 8 x 10^6 nauplii a month or 4 x 10^6 nauplii a tank each month. Aquacop (1979) estimated that one spawning a month for each female can be achieved, producing a minimum of 4 x 10^6 eggs and 2 x 10^6 nauplii.

There were high mortalities among the Mexican strain of *P. stylirostris* in the systems used during this study. It is believed that the combination of shipping techniques (in chilled vermiculite) and acclimation techniques resulted in the high mortalities as well as lesions of the exoskeleton. It was necessary for the Mexican strain of *P. stylirostris* to molt at least two to three times before maturation and spawning because of the lesions on the exoskeleton. The appearance of the animals improved greatly after two molts. The unablated and the ablated females matured and spawned producing 2 x 10^6 eggs and 8.35 x 10^6 nauplii and 5.4 x 10^6 eggs and 3.3 x 10^6 nauplii, respectively. It is significant that the *P. stylirostris* (unablated) matured and spawned as well as the ablated shrimp, suggesting that unilateral eyestalk ablation is not a required procedure, but it appears to accelerate initial ovarian development and later redevelopment of the ovaries after prior spawning.

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